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Examining Group 185

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Sherie L. Morrison, et al.
Serial No. : 07/675,106
Filed : March 25, 1991
For : RECEPTORS BY DNA SPLICING
AND EXPRESSION
Group : 185
Examiner : T. Nisbet

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New York, New York
January 3, 1991

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

AMENDMENT

Sir:

Kindly amend the application as follows:

IN THE CLAIMS

Please cancel, without prejudice, claim 42.

Kindly amend the pending claims as follows:

39. (Twice amended) A method for producing a functional antibody [receptor] having two subunits [two-subunits], which comprises the steps of:

(a) transfecting a non-antibody producing lymphoid [mammalian] cell with a first DNA sequence coding for a first subunit of the antibody [receptor];

(b) transfecting the cell with a second DNA sequence, said second DNA sequence coding for a second subunit of the antibody [receptor], said second subunit being a subunit other than the first subunit; and

(c) maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA sequences and the resultant subunits are intracellularly assembled [bound] together to form the antibody which is then secreted

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in a form capable of specifically binding to antigen [a receptor].

43. A method as recited in claim 39, [42] wherein the cell is a myeloma cell.

54. A method for producing a functional antibody [receptor] having two subunits, which comprises the steps of:

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(a) transfecting a non-antibody producing lymphoid [mammalian] cell with a plasmid comprising a first DNA sequence coding for a first subunit of the antibody [receptor] and a second DNA sequence coding for a second subunit of the antibody [receptor], said second subunit being a subunit other than the first subunit; and

(b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA sequence and said second DNA sequence and the resultant subunits are intracellularly assembled [bound] together to form the antibody which is then secreted in a form capable of specifically binding to antigen [a receptor].

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Please add the following claim:

55. A method as recited in claim 39 wherein the antibody is a chimeric antibody having a variable region substantially the same as that found in a first mammalian source and having a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

REMARKS

Applicants first express their thanks to the Examiner for allowing them an interview. Applicants have amended the claims to more particularly recite the subject matter of the present invention in the manner discussed with the Examiner during the interview.

The present claims as amended are fully supported by the specification and fully meet the requirements of 35 U.S.C. § 112. The claims are directed to producing a functional antibody having two subunits by co-transforming a non-antibody producing lymphoid cell. These antibodies contain constant and variable regions.

The claims are no longer drawn to cover "receptors". Thus, the basis for previous rejections that the application is not enabled for multi-unit receptors (e.g. T-cell receptors and Major Histocompatibility Complex antigens) has been removed. The present claims as amended are directed to a method of producing antibodies, which have variable and constant regions, and are fully supported by the specification.

Claims 39-48 and 52-54 stand rejected under 35 U.S.C. § 103 as obvious over Cabilly or Boss in view of Gillies. Applicants respectfully traverse this rejection. Applicants' invention, as claimed, consists of co-transforming a non-immunoglobulin producing lymphoid cell to obtain expression of functional antibodies. This invention was not obvious in view of the prior art.

As explained in detail in Applicants' May 15, 1991 Supplemental Preliminary Amendment, the work described in Cabilly was based exclusively on experiments done in bacterial cells. Although co-transformation in bacterial systems is disclosed, Cabilly did not express a preference for co-transformation over combining the extracts from

singly-transformed cells. In fact, the Wetzel affidavit submitted during prosecution of the Cabilly patent (copy submitted with applicants' May 15, 1991 paper) expressly states that "the results with the co-transformant extracts and combined extracts were essentially the same". (Wetzel Aff., p. 2). And, of course, Cabilly's approach did not result in the direct production of antibodies. Cabilly requires recombination of heavy and light chains to form a minute level of antibody.

Moreover, Cabilly does not mention or suggest the use of non-antibody producing lymphoid cells. Cabilly's token reference to mammalian cells, discussed at page 8 of applicants' May 15, 1991 paper, is not based on experimental work and does not even suggest co-transforming non-antibody producing lymphoid cells to express functional antibodies.

Gillies does not teach co-transformation. What Gillies discloses is transforming a J558L heavy chain loss mouse myeloma cell with an exogenous heavy chain gene. The heavy chain formed a tetramer with an endogenous light chain. Apart from the fact that Gillies did not teach co-transformation of exogenous genes, the prior art on a whole taught that the exogenous-endogenous approach of Gillies would not always work. In Oi (discussed at pages 4-5 of applicants' May 15, 1991 paper) the results were inconsistent. A mouse myeloma cell supported expression of a transfected immunoglobulin gene. However, a rat myeloma cell line which synthesizes and secretes a rat kappa light chain, when treated in the same manner as the mouse myeloma did not support expression of a transfected immunoglobulin chain gene, even though the cell did produce an endogenous light chain. Oi stated in this regard that

"Further studies are needed to determine the cause of this variation and why light chain expression does not occur in

Y3 or BW5147 cell lines transformed with the same light chain gene vector."
(p. 828)

In light of this, it would not have been obvious that co-transforming exogenous genes in the host cells used by Gillies and Oi would have resulted in the expression of functional antibody. Functional antibodies had never been obtained by co-expressing exogenous immunoglobulin genes in a lymphoid cell. Taken in the aggregate, the prior art in no way provided applicants with a reasonable expectation of success when they undertook to co-transform and co-express both light and heavy chains in non-immunoglobulin producing lymphoid cells in order to obtain functional antibodies. Consequently, applicants' invention is not obvious in view of the prior art.

In view of the uncertainty in the prior art regarding the success of the exogenous-endogenous approach, it would have not been obvious to try the approach of exogenous co-transformation and co-expression in lymphoid cells that the prior art did not disclose. Furthermore, "obvious to try is not the standard of 35 U.S.C. § 103." In Re Yates, 663 F.2d 1054, 1057 (1981). The CCPA has stated:

"Slight reflection suggests, we think, that there is usually an element of 'obvious to try' in any research endeavor, that it is not undertaken with complete blindness but rather with some semblance of a chance of success, and that patentability determinations based on that as the test would not only be contrary to statute but result in a marked deterioration of the entire patent system as an incentive to invest in those efforts and attempts which go by the name of 'research'."

Application of Tomlinson, 363 F.2d 928, 931 (1966).

The Federal Circuit requires the evaluation of secondary considerations before the issue of obviousness is decided. Stratoflex, Inc. v. Aeroquip Corp., 713 F.2d 1530, 1538 (Fed. Cir. 1988). During the interview, the secondary

consideration of unexpected results was reviewed. As discussed at the interview, applicants' invention unexpectedly results in the expression of functional antibodies at significant binding levels. This is in sharp contrast to the negligible levels of antibody activity disclosed in Cabilly.

As set forth in applicants' May 15, 1991 Supplemental Preliminary Amendment at pp. 6-8, Cabilly's bacterial double transformant approach required "recombination" of light and heavy chains. This resulted in an extremely low level of antibody formation -- 0.76% of the chains are reported as having "recombined" to form antibody. Third parties skilled in the art have pointed out the failure of the bacterial approach to produce useful or significant amounts of antibodies with specific binding ability. Arne Skerra and Andreas Pluckthun state that:

"In *E. coli*, the antibody protein could be produced only in a non-native state[], and refolding experiments led to only a small percentage of correctly folded recombinant antibodies." Science, 240, pp. 1038-1041 at p. 1038 (May 20, 1988) (emphasis added).

Similarly, Antonino Cattaneo and Michael S. Neuberger cite to Cabilly and comment that:

"the introduction of vectors driving expression of immunoglobulin cDNAs into yeast or *Escherichia coli* hosts has not resulted in effective antibody production--problems being encountered both with efficient assembly and with secretion (Cabilly et al., 1983; Boss et al., 1984; Wood et al., 1985)." EMBO J., 6, pp. 2753-58 at p. 2753 (1987).

In contrast, applicants achieved significant binding antibody production by co-transforming non-antibody producing lymphoid cells. As disclosed in the specification:

"When transfected J558L cells producing the human Ig G2(k) chimeric anti-PC antibody were grown as a subcutaneous

tumor in BALB/c mice, analysis of the sera of these mice showed significant human Ig G2(k) anti-PC binding antibody produced by radioimmunoassay. Polyclonal anti-human antiserum demonstrated the present of significant quantities of human immunoglobulin in the sera." (pg. 19, lines 30-37)

Functionality was also established by phosphocholine binding:

"Phosphocholine-binding by the chimeric antibody produced in the J558L cell line was the result from specific association of the chimeric immunoglobulin light and heavy polypeptide chains....This was determined by measuring PC-binding by immunoglobulins produced by J558L cells transfected with the mouse:human chimeric heavy chain gene." (p. 18, lines 24-32)

Applicants also included evidence that the binding specificity of their antibodies is due to their expression initially in correctly folded form such that antigen-specific binding sites exist without need of further processing.

"Three monoclonal anti-idiotope antibodies, each recognizing a distinct epitope on the light and heavy variable region domains and an epitope defined by the presence of both light and heavy variable region domains, were found to react with the mouse:human chimeric anti-PC antibodies. This strongly supports the fact that the mouse S107 antigen-binding domains have folded into their intended structures." (p. 19, lines 7-15)

The production of significant and useful amounts of antibodies capable of specific antigen binding is central to applicants' invention. Specificity and its attendant advantages are discussed throughout applicants' specification. The first sentence in the Description of the Specific Embodiments begins, "[n]ovel methods and compositions are provided[] for production of polypeptide products having specific binding affinities for a predetermined ligand...."(p. 2, lines 32-34)

In contrast to applicants' focus on the binding functionality of the antibodies produced by the method of the claimed invention, Cabilly discloses only very limited and slim support for its claim of antigen binding (col. 26, line 62 - col. 27, line 27). Cabilly does not disclose or enable the production of functional antibodies on a significant scale, as achieved by applicants. Rather, Cabilly reported a minute yield of "recombined" antibody (Cabilly patent, col. 27, lines 18-27).

Cabilly's 0.76% yield is based on an estimate of the levels of heavy and light immunoglobulin chains in the reaction mixtures and on an antigen binding assay. Not only is this calculated yield dubious by virtue of its reliance on an estimate with unknown associated error, but the calculation also used as a background number the binding measured for cells producing light chain only. The background number may have been higher if the antigen binding for cells producing only heavy chains had been measured. (In fact, it has been found that heavy chains alone will bind antigen in the absence of a complementary light chain. Ward et al., Nature, vol. 341, pp. 544-46 (1989).) This would have resulted in an even lower percent recombination. Even given this potential for overestimating percent recombination, Cabilly calculated obtaining in active form only a fraction of one percent of the antibody protein.

Boss is not available as a prior art reference against any claim of the present application. The § 102(e) date of the Boss patent is November 14, 1984. The present application claims the benefit of the August 27, 1984 filing date of United States patent application 644,473. More specifically, the present application is a continuation of United States patent application 441,189, filed November 22,

1989 as a continuation of United States patent application 644,473, filed August 27, 1984. The 441,189, 090,669 and 644,473 applications are now abandoned. As compared with the specification of the 644,473 application, the additional material included in the present application disclosed and enabled intra-species chimeric receptors. The specific claims that rely on that additional material for support have been cancelled in the application. All the remaining claims are directed to the expression of functional antibodies by co-transforming non-antibody producing lymphoid cells as disclosed in the original specification and are fully supported by the specification as originally filed in the 644,473 parent application. Accordingly, the Boss patent is not prior art to the pending claims of this application under 35 U.S.C. § 102(e) or, consequently, under 35 U.S.C. § 103.

Applicant makes of record Sharon et al., Nature, vol. 309, pp. 364-367 (May 24, 1984). The reference does not disclose exogenous co-transformation and co-expression in a non-antibody producing lymphoid cell to produce a functional antibody. Sharon discloses transforming a J558L mouse myeloma heavy chain loss variant cell with a single chimeric immunoglobulin chain gene having a heavy variable region and a light constant region. The single chimeric chain that was produced was then associated in vitro with a separately expressed light chain produced in another cell. This is not a disclosure of co-transformation and co-expression in a single cell to produce a functional antibody. Sharon also discloses an exogenous-endogenous approach in which the chimeric gene is transfected into a myeloma cell which is subsequently fused with a cell that endogenously expresses the light chain having the complimentary variable region resulting in an in vivo

association of the chains. This too is not a disclosure of exogenous co-transformation as claimed by applicants.

Alternatively, applicants submit that Sharon is not prior art to their invention. As set forth in the Morrison and Oi Declarations* that are submitted with this Amendment under 37 C.F.R. § 1.131, applicants' invention was conceived and reduced to practice prior to the May 24, 1984 publication date of Sharon.

Applicants also make of record Ochi et al., Proc. Natl. Acad. Sci. USA, vol. 80, pp. 6351-6355 (October 1983). This reference, which we have been advised was mailed to subscribers on October 19, 1983, is not prior art to applicants' invention. The Morrison and Oi Declarations submitted with this Amendment under 35 C.F.R. § 1.131 establish conception of applicants' invention prior to the effective date of the reference coupled with due diligence from prior to said date to a subsequent reduction to practice.

In view of the foregoing, applicants believe that the amended claims are in condition for allowance. Reconsideration of the application and allowance of the claims are requested.

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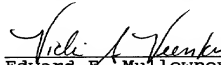
JANUARY 3, 1992

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* The Morrison and Oi Declarations are submitted herewith in telecopied form. The originals of those declarations bearing the inventors' signatures will be submitted to the Patent Office as soon as they are received by the undersigned attorneys.